

In re Application of:

Frost et al.

Application No.: Not Yet Assigned

US Submission Date: June 13, 2005

Based on Intl Appl: PCT/US2003/040090

IA Filing Date: December 15, 2003

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## **B. In the Claims**

Please amend claim 23 without prejudice.

Upon entry of the present amendment, the claims will stand as follows in the present application:

1. (original) A substantially purified chondroitinase glycoprotein comprising, a CHASEGP polypeptide and at least 1 N-linked sugar moiety, wherein said N-linked sugar moiety is covalently attached to an asparagine residue of said polypeptide.

2. (original) The glycoprotein of claim-1, wherein the polypeptide is selected from the group of a polypeptide that comprises a sequence of amino acids encoded by nucleotides 642-2087 in SEQ ID No. 3 and includes at least about 74% amino acid sequence identity with the sequence of amino acids set forth in SEQ ID No. 1; a polypeptide that comprises a sequence of amino acids encoded by the sequence of nucleotides set forth in SEQ ID No. 2; a polypeptide that comprises a sequence of amino acids encoded by a sequence of nucleotides that hybridizes along at least 85% of its full-length under conditions of high stringency to the sequence of nucleotides set forth as nucleotides 642-2087 in SEQ ID No. 3.

3. (original) The glycoprotein of claim-1, wherein said sugar moiety is covalently attached to an asparagine residue selected from the group in SEQ ID No. 1 comprising amino acid number's 86, 115 and 343.

4. (original) The glycoprotein of claim-1, wherein said sugar moiety is covalently linked to said glycoprotein through a PNGase sensitive bond.

5. (original) The glycoprotein of claim-1, wherein said sugar moiety is of the high mannose type.

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6. (original) The glycoprotein of claim-1, wherein said sugar moiety is of the complex type.

7. (original) The glycoprotein of claim-1, wherein said sugar moiety is of the hybrid type.

8. (original) The glycoprotein of claim-1, wherein said sugar moiety is substantially terminated with sialylic acid.

9. (original) A substantially purified glycoprotein of claim-1, wherein said CHASEGP portion of the polypeptide consists essentially of the chondroitinase domain of the CHASEGP or a catalytically active portion thereof.

10. (original) The substantially purified glycoprotein of claim 1, wherein the chondroitinase domain comprises the sequence of amino acids set forth as amino acids 35-457 of SEQ ID No. 1.

11. (original) The substantially purified glycoprotein of claim 1 that has more than about 80% sequence identity with a polypeptide that comprises the sequence of amino acids set forth as SEQ ID No. 1 or as the sequence of amino acids set forth as SEQ ID No. 2, wherein the polypeptide is a chondroitinase.

12. (original) A polypeptide of claim 1, wherein the chondroitinase domain portion is encoded by a nucleic acid molecule that hybridizes under conditions of high stringency along at least 70% of its full-length to a nucleic acid molecule comprising a sequence of nucleotides set forth as nucleotides 726-1995 in SEQ ID No. 3 or as SEQ ID No. 5. or at least one domain thereof or a catalytically active portion of the domain.

13. (original) The substantially purified glycoprotein of claim 1, wherein the CHASEGP is a human polypeptide.

14. (original) A glycoprotein of claim-1, wherein said CHASEGP polypeptide encodes a soluble polypeptide as described in SEQ ID NO. 6.

15. (original) A glycoprotein of claim 1, wherein the chondroitinase domain portion is encoded by a nucleic acid molecule that hybridizes under conditions of high stringency along at least 70% of its full-length to a nucleic acid molecule comprising a sequence of nucleotides set forth as nucleotides 726-1995 in SEQ ID No. 3 or as SEQ ID No. 5 or at least one domain thereof or a catalytically active portion of the domain.

16. (original) The glycoprotein of claim 1, wherein: the polypeptide does not comprise the complete sequence set forth in SEQ ID No. 1 and includes at least amino acids 35 to 264 of SEQ ID 1.

17. (original) A glycoprotein of claim 1 that is a mutein, wherein: up to about 50% of the amino acids are replaced with another amino acid; and the resulting polypeptide is a single chain or two chain polypeptide that has catalytic activity of at least 10% of the unmutated polypeptide.

18. (original) The glycoprotein of claim 17, wherein up to about 10% of the amino acids are replaced with another amino acid.

19. (original) The glycoprotein of claim 17, wherein the resulting polypeptide is a single chain or two chain polypeptide and has catalytic activity of at least 50% of the unmutated polypeptide.

20. (original) The glycoprotein of claim 17, wherein a free Cysteine in the chondroitinase domain is replaced with another amino acid

21. (original) The glycoprotein of claim 20, wherein the replacing amino acid is a serine.

22. (original) An isolated substantially pure glycoprotein that consists essentially of the chondroitinase domain of CHASEGP.

23. (currently amended) A nucleic acid molecule, comprising a sequence of nucleotides that encodes the polypeptide of [[any of claims 1-21]]claim 1.

24. (original) The nucleic acid molecule of claim 23 that comprises a sequence of nucleotides selected from the group consisting of: (a) a sequence of nucleotides set forth as nucleotides 726-1995 in SEQ ID No. 3; (b) a sequence of nucleotides that hybridizes under high stringency along its length or along at least about 70% of the full-length to the sequence of nucleotides set forth as nucleotides 726-1995 in SEQ ID No. 3 or as SEQ ID No. 5 (c) a sequence of nucleotides that encodes the polypeptide of SEQ ID No. 6; (d) a sequence of nucleotides that is a splice variant of a, b, or c); (e) a sequence of nucleotides that encodes the chondroitinase domain or a catalytically active portion thereof that includes a sequence of nucleotides having at least about 60%, 70%, 80%, 90% or 95% sequence identity the sequence set forth in SEQ ID Nos. 3,4 or 5; and (f) a sequence of nucleotides comprising degenerate codons of (a), (b),(c), (d) or (e).

25. (original) An isolated nucleic molecule that encodes a mutein of claim 17.

26. (original) A vector comprising the nucleic acid molecule of claim 23.

27. (original) The vector of claim 26 that is an expression vector.

28. (original) The vector of claim 26 that is a eukaryotic vector.

29. (original) The vector of claim 26 that includes a sequence of nucleotides that directs secretion of any polypeptide encoded by a sequence of nucleotides operatively linked thereto.

30. (original) The vector of claim 26 that is a Pichia vector or an E. coli vector.

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31. (original) A cell, comprising the vector of claim 26.
32. (original) The cell of claim 31 that is a prokaryotic cell.
33. (original) The cell of claim 31 that is a eukaryotic cell.
34. (original) The cell of claim 31 that is selected from among a bacterial cell, a yeast cell, a plant cell, an insect cell and an animal cell.
35. (original) The cell of claim 31 that is a mammalian cell.
36. (original) A nucleic acid molecule encoding a polypeptide of claim 1.
37. (original) A vector, comprising nucleic acid molecule of claim 23.
38. (original) A cell, comprising the vector of claim 23.
39. (original) A recombinant non-human animal, wherein an endogenous gene that encodes a polypeptide of claim 1 has been deleted or inactivated by homologous recombination or insertional mutagenesis of the animal or an ancestor thereof.
40. (original) A method for generating soluble recombinant CHASEGP comprising, introduction of a nucleic acid as described in SEQ ID NO: 4 operably linked to a suitable promoter into a eukaryotic cell capable of incorporating said N-linked sugar moieties into CHASEGP.
41. (original) The method of claim 40, wherein the eukaryotic cell is mammalian.
42. (original) The method of claim 40, wherein said eukaryotic cell is an insect.
43. (original) The method of claim 40, wherein said eukaryotic cell is a yeast
44. (original) The method of claim 3, wherein said eukaryotic cell is a plant.

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45. (original) The method of claim 40, wherein the expressible polynucleotide is introduced into a cell ex vivo, thereby generating a genetically modified cell containing the expressible polynucleotide, and wherein administering the expressible polynucleotide to the subject comprises administering the genetically modified cell to the subject.

46. (original) The method of claim 45, wherein the cell is autologous with respect to the subject.

47. (original) The method of claim 45, wherein the cell is haplotype matched with respect to the subject.

48. (original) A method for generating the CHASEGP comprising, contacting chondroitinase polypeptide of claim 1 with glycosyltransferase enzymes capable of introducing said N-linked sugar moieties to generate CHASEGP.

49. (original) The method of claim 48 wherein the glycosyltransferase enzymes are derived from canine microsomal membranes.

50. (original) A composition, comprising a substantially purified CHASEGP glycoprotein in conjunction with a suitable pharmaceutical carrier.

51. (original) A method for treating an animal suffering from an excess of CHASEGP substrate, said method comprising administration of a recombinant CHASEGP in an amount sufficient to remove said CHASEGP substrate.

52. (original) The method of claim 51, wherein said excess substrate is produced from a scar tissue.

53. (original) The method of claim 52, wherein said scar tissue is a glial scar resulting from spinal cord injury.

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54. (original) The method of claim 52, wherein said scar tissue is a result of surgery.

55. (original) The method of claim 52, wherein said scar is a keloid scar.

56. (original) The method of claim 51 wherein said substrate is associated with a herniated disk.